Seminars in Cancer Biology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cancer Biology

journal homepage: www.elsevier.com/locate/semcancer

Review

HTLV-1 clonality in adult T-cell leukaemia and non-malignant HTLV-1 infection

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ARTICLE INFO

Keywords:
HTLV-1
Retrovirus
Leukaemia
Lymphoma
Persistent infection
Clonality
Integration
High-throughput sequencing

ABSTRACT

Human T lymphotropic virus type 1 (HTLV-1) causes a range of chronic inflammatory diseases and an aggressive malignancy of T lymphocytes known as adult T-cell leukaemia/lymphoma (ATLL). A cardinal feature of HTLV-1 infection is the presence of expanded clones of HTLV-1-infected T cells, which may persist for decades. A high viral burden (proviral load) is associated with both the inflammatory and malignant diseases caused by HTLV-1, and it has been believed that the oligoclonal expansion of infected cells predisposes to these diseases. However, it is not understood what regulates the clonality of HTLV-1 in vivo, that is, the number and abundance of HTLV-1-infected T cell clones. We review recent advances in the understanding of HTLV-1 infection and disease that have come from high-throughput quantification and analysis of HTLV-1 clonality in natural infection.

1. Introduction

Human T lymphotropic virus type 1 (HTLV-1) is a retrovirus that is widespread in the tropics and sub-tropics. The total number of people infected is at least 5 to 10 million, but the true number is very uncertain, owing to incomplete epidemiological studies in the endemic regions [1].

HTLV-1 and its congeners HTLV-2, 3 and 4 [2] are retroviruses that belong to the *Deltaretrovirus* genus of the subfamily *Orthoretrovirinae*, while the other pathogenic human retroviruses HIV-1 and 2 are classified in the subfamily *Lentivirinae*. Unlike HIV-1 and 2, HTLV-1 does not cause disease in the majority (over 90%) of infected individuals. Between 1 and 4% of HTLV-1-infected people develop a chronic inflammatory disease, of which the commonest is HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), which causes progressive paralysis of the legs [3]. Some 5% of HTLV-1-infected individuals develop adult T cell leukaemia/lymphoma (ATLL), a T cell malignancy with a characteristically poor prognosis [4].

The history [5,6] and epidemiology [1,7,8] of HTLV-1 have been ably reviewed elsewhere. The purpose of the present review is to consider two questions. First, what regulates the clonality of HTLV-1 in vivo, that is, the selective outgrowth of certain clones of T cell infected with HTLV-1? Second, what is the role of this oligoclonal proliferation in the pathogenesis of the inflammatory and malignant diseases associated with HTLV-1? A clone of HTLV-1-infected T cells is identified as a population of cells that carry the HTLV-1 provirus integrated at the same site in the host genome.

2. Adult T cell leukaemia/lymphoma (ATLL)

ATLL is a malignancy of mature, post-thymic T lymphocytes [9]. ATLL cells have a characteristic morphology, with a large, multi-lobed nucleus, giving rise to the epithet "flower cell". In the great majority of cases, the phenotype of the malignant cell is $CD4^+$ $CD8^-$; about 4% of cases are $CD4^-$ $CD8^+$, and a similar proportion $CD4^+$ $CD8^+$ or $CD4^-$ $CD8^-$ [10]. The cells usually express the markers CD2 and CD5; CD3 and TCRß are frequently downregulated or undetectable at the cell surface. The cells also express several molecules that are characteristic of regulatory T cells, including the cell surface molecules CD25, CCR4, GITR and the transcription factor FoxP3. However, these molecules are also expressed by activated T cells, and it appears that ATLL is not per se a malignancy of regulatory T cells [11].

ATLL was classified into 4 clinical subtypes by Shimoyama et al. [12], according to the lymphocyte count, serum calcium concentration, lactate dehydrogenase level, solid organ involvement and the severity of systemic symptoms. The most common acute form (about 65% of cases) can present as a medical emergency, with bulky lymphadenopathy, a florid and rapidly increasing leukocytosis, hypercalcaemia, frequently with destructive bone lesions, dehydration, and severe systemic symptoms. In the chronic form, the lymphocytosis can also be very marked (over 50×10^9 cells L^{-1}), but the cell count rises more slowly, and the patient can remain stable with minor or absent symptoms for months or even years. A proportion of cases (~20%) present as a lymphoma, with a

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1044-579X/$ – see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.semcancer.2013.11.003

normal circulating lymphocyte count. This diagnostic classification remains useful for purposes of standardizing clinical trials, comparing disease and treatment outcomes between centres, choosing appropriate therapy and for assessing the prognosis. However, the classification does not reflect the continuum of presentation in the clinic. For example, a purely cutaneous form of ATL lymphoma is recognized, which occurs without leukaemic or nodal disease, and which carries a substantially better prognosis than nodal lymphomas.

3. Treatment

ATLL carries a poor prognosis because of intrinsic chemotherapy resistance and severe immunosuppression. Despite advances in medical management and supportive care, chemotherapy trials report a median survival of the aggressive subtypes between 7 and 13 months [13–15]. Clinical trials of combination chemotherapy in acute ATLL have achieved improved response rates but have not prolonged survival. Patients with indolent forms of ATLL have a better prognosis (median overall survival 4.1 years [16]) but the long-term survival remains poor when managed with either watchful waiting or conventional chemotherapy. A recent meta-analysis of non-Japanese patients treated with zidovudine and IFNα revealed this to be a highly effective treatment for leukaemic subtypes of ATLL [17]. Lymphoma subtypes may still benefit from chemotherapy, with either concurrent or sequential zidovudine + IFNα treatment to prevent relapse [18]. The risk of relapse with all ATLL subtypes remains high and the role of consolidation treatment with immunomodulatory therapies such as zidovudine + IFNα, arsenic trioxide or with monoclonal antibodies such as basiliximab or mogamulizumab is yet to be established. Allogeneic bone marrow transplantation remains the only curative option but is only possible in those individuals who achieve a complete response to treatment, have an HLA-matched donor and are physically fit for the procedure.

4. HTLV-1 molecular virology

The genome of HTLV-1 and the major transcripts are shown in Fig. 1. In addition to the *gag*, *pol* and *env* gene products found in other exogenous replication-competent retroviruses, HTLV-1 encodes at least 7 regulatory gene products which control the proviral transcription, mRNA splicing and transport, and the expression of certain host genes. The functions of these regulatory genes of HTLV-1 have been reviewed elsewhere [19,20]. Among these genes, two, *tax* and *HBZ*, appear to play a particularly important role in regulating the expression of viral and host genes and the activation and proliferation of the host cell [20,21]. The transcriptional transactivator Tax recruits host cell transcription factors, notably CBP/p300, and activates transcription of the virus itself, from the promoter/enhancer in the 5′ long-terminal repeat (LTR) (Fig. 1), creating a strong positive feedback loop. In addition, Tax activates the NF-κB and AKT pathways, thereby upregulating many host genes [22]. This widespread gene activation results in activation and proliferation of the host cell [20,23] and transmission of HTLV-1 to other host cells via the virological synapse [24,25].

HTLV-1 Tax protein has a remarkable range of actions on the host cell, promoting DNA replication and cell-cycle progression, structural damage to the host cell DNA, inhibition of DNA repair and cell-cycle and DNA damage checkpoints, and centrosome overduplication. Understandably, Tax has therefore been believed to be necessary and sufficient to cause ATLL. Tax is indeed sufficient to immortalize rat fibroblasts in culture, and Tax-transgenic mice develop a variety of tumours [26–28]. However, mouse cells appear to be transformed more readily than human cells [29], and attempts to transform human cells in vitro with Tax have failed.

A second paradox concerning the putative oncogenic role of Tax is the fact that some 60% of ATLL clones do not express Tax, although the transformed cell typically retains the phenotype ($CD25^+$ $FoxP3^+$ $GITR^+$, etc.) of the Tax-expressing cell. The loss of Tax results from one of 3 mechanisms: deletion or methylation of the 5′ LTR, or mutation of the provirus [20,21]. It is thought that the pressure to lose Tax expression is exerted by the strong host cytotoxic T lymphocyte (CTL) response to the Tax protein [30].

In 2002 a new gene was discovered in HTLV-1 [31]. The HTLV-1 bZIP factor, HBZ, is expressed from the negative strand of the provirus (Fig. 1), driven by the transcription factor Sp1 from a promoter in the 3′ LTR. In contrast with Tax, HBZ appears to be expressed at a constant (albeit low) level in most if not all HTLV-1-infected cells, both non-transformed and malignantly transformed [32].

HBZ has important actions at both the protein and mRNA levels [20]. HBZ protein can reduce Tax-mediated viral transcription by heterodimerizing with Jun and CREB2 [33]. HBZ also selectively inhibits activation of the classical NF-κB pathway [34]; since Tax activates both the classical and alternative pathways of NF-κB, it is possible that chronic activation of the alternative NF-κB pathway by persistent HBZ expression plays a part in the proliferation of ATLL cells in vivo [20]. This interpretation is favoured by the observation that an efficient $CD8^+$ T-cell response to HBZ is associated with a lower proviral load and a lower risk of the inflammatory disease HAM/TSP [35,36]. HBZ mRNA, rather than the protein, promotes expression of the transcription factor E2F1, supports proliferation of ATLL cells in vitro [32], increases the proviral load of HTLV-1 in the rabbit [37], and increases the activity of the telomerase hTERT [38].

5. Cellular tropism and propagation of HTLV-1

HTLV-1 can infect virtually all nucleated mammalian cells in vitro [39], but in vivo it is almost confined to T lymphocytes and dendritic cells (DCs) [25,40]. Typically about 95% of the proviral load – the proportion of circulating mononuclear leukocytes infected – is carried in $CD4^+$ (helper/regulatory) T cells, and 5% in $CD8^+$ T cells [40] (AM, unpublished data). DCs constitute a very small fraction of the load, but it is possible that they play a disproportionate role in propagating the virus within one host, particularly in the early stages of infection, because of their high mobility and their propensity to form intimate contacts with other cells [41,42]. HTLV-1 releases almost no cell-free virus particles in vivo. Instead, when an infected cell makes contact with another cell, a synergistic interaction between extracellular and intracellular signals leads to cytoskeletal polarization in the infected cell and causes directed assembly and budding of the virus at the cell-to-cell contact, resulting in efficient transfer of the virus to the "target" cell [24]. This specialized, virus-induced cell-to-cell contact is known as a virological synapse [24]. Thus, the virus exploits the mobility of the host cell instead of releasing mobile extracellular particles. As a result, cell-free blood products from HTLV-1-infected people are not infectious; HTLV-1 is transmitted between individuals by transfer of infected leukocytes in breast milk, semen or blood [7].

5.1. What determines the equilibrium proviral load in an individual with HTLV-1 infection?

Early studies found no systematic association between HTLV-1 genotype and disease manifestation [43–45]. In 2000, Furukawa and his colleagues reported [46] a higher prevalence of HAM/TSP among people in southern Japan infected with the cosmopolitan

Please cite this article in press as: Bangham CRM, et al. HTLV-1 clonality in adult T-cell leukaemia and non-malignant HTLV-1 infection. Semin Cancer Biol (2013), http://dx.doi.org/10.1016/j.semcancer.2013.11.003